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Formal taxonomy of species C of the *Anopheles minimus* sibling species complex (Diptera: Culicidae)

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Abstract

The nomenclatural identity of species C of the *Anopheles minimus* complex is resolved by excluding the available junior synonyms of the nominotypical member of the complex and naming it *An. harrisoni* Harbach & Manguin, **sp. n.** *Anopheles formosaensis I* Tsuzuki, *An. christophersi* Theobald and *An. christophersi* var. *alboapicalis* Theobald are retained as junior synonyms of *An. minimus* Theobald based on the provenance of type specimens in geographical areas where *An. harrisoni* is not known to occur. A lectotype is designated for *An. vincenti* Laveran, which thus becomes the senior name of the specific entity known as *An. jeyporiensis* James. Molecular data that diagnose *An. harrisoni* are reviewed and the holotype female is contrasted with the neotype series of *An. minimus*. Available information on the bionomics and distribution of the new species is included.

Key words: Mosquito, *minimus* C, Minimus Complex, *Anopheles harrisoni*, new species

Introduction

Anopheles minimus Theobald is the nominotypical member of a sibling species complex (Minimus Complex) of malaria vectors in the Oriental Region. The taxonomic history of the complex was reviewed by Chen *et al*. (2002) and Harbach *et al*. (2006). As currently defined, the complex includes three genetic species of the Minimus Subgroup within the Funestus Group (Garros *et al*., 2005b) that are informally denoted in literature as species A, C and E (Harbach, 2004; Somboon *et al*., 2001). Species A is widespread in the Oriental Region, species C has a disjunctive distribution in Southeast Asia, and species E is known only from Ishigaki Island of the Ryukyu Archipelago, Japan (Somboon *et al*., 2001, 2005a).

Anopheles minimus was named and described by Theobald (1901) from a single female that became nonextant after 1907 (Harrison, 1980). To fix the identity of this species and provide a foundation for further taxonomic studies of the Minimus Complex, Harbach *et al*. (2006) selected a neotype from specimens collected near the original type locality in Hong Kong. Sequences for the D3 domain of the 28S locus of ribosomal DNA (rDNA) and the cytochrome oxidase subunit II locus (COII) of mitochondrial DNA (mtDNA) obtained from a hindleg of the neotype confirmed its identity as species A. Consequently, *An. minimus* species A is *An. minimus s.s*.

Anopheles minimus and species C are partially, albeit dubiously, distinguished by the presence of a humeral pale spot (HP) on the wings. Green *et al*. (1990) found that this spot was present in 78% of species C

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whereas it was present in only 5% of *An. minimus* (as species A) females from Kanchanaburi Province in western Thailand. Similarly, Sharpe (1997) recorded the presence of a HP spot in 63% of species C as opposed to 9% of *An. minimus* (as species A) collected at the same locality (Ban Phu Rat) visited by Green *et al*. (1990). Chen *et al*. (2002) noted the presence of this spot in a comparable percentage of *An. minimus* females (7.3%, as species A) from southern China, but it was present in significantly fewer specimens of species C females (15.6%).Van Bortel *et al*. (1999) observed an even higher degree of similarity between the two species in northern Vietnam where 91.8% of species C (as form II) and 99% of *An. minimus* (as form I) lacked HP spots. Finally, Sungvornyothin *et al*. (2006a) provided similar data on the occurrence of HP spots in populations of *An. minimus* (as species A) and species C from sites in Kanchanaburi and Tak provinces in western Thailand. From these studies, it is obvious that the presence or absence of HP spots cannot be used as a diagnostic character to identify or distinguish the two species with any degree of confidence. Van Bortel *et al*. (1999), Chen *et al*. (2002) and Sungvornyothin *et al*. (2006a) also examined the presence/absence of a presector pale spot (PSP) on the wings of males and females and showed that this character is even less reliable for distinguishing the two species. To date (see below), no morphological characters have been found in any life stage to distinguish the two species.

Garros *et al*. (2005b) and Chen *et al*. (2006) regarded *An. fluviatilis* species S of northern India as a synonym of *An. minimus* species C based on the homology of the D3 region of 28S rDNA, which extended the distribution of the latter species into India. More recently, however, the occurrence of *An. minimus* C in India was refuted by Singh *et al*. (2006) who showed that *An. fluviatilis* S is distinct from *An. minimus* C based on appreciable differences in the sequences of the second internal transcribed spacer (ITS2) locus and D2-D3 domain of 28S rDNA. Singh *et al*. (2006) also documented the presence of species C in central Myanmar.

Harbach (2004) pointed out that little progress has been made in giving formal names to members of sibling species complexes in cases where the availability of junior synonyms of the nominotypical species must be considered. This is undoubtedly the principal reason why species C of the Minimus Complex has not been given a formal name. The process of determining whether an available name may apply to a cryptic species is complicated by the lack of diagnostic features and DNA sequence data for type specimens.

The names of four nominal species (one with an unjustified replacement name, see Harbach *et al*., 2006) are currently regarded as junior synonyms of *An. minimus*. Three of these names undoubtedly denote the same specific entity as the neotype of *An. minimus* from Hong Kong. These include *An. formosaensis I* Tsuzuki, *An. christophersi* Theobald and *An. christophersi* var. *alboapicalis* Theobald. Tsuzuki (1902) described *An. formosaensis I* from adult mosquitoes collected at an undisclosed location on the island of Taiwan, and *An. christophersi* and the variety *alboapicalis* were described by Theobald (1902 and 1910, respectively) from specimens collected at localities in the Duars region of western Assam, India. Molecular data show that species C does not extend as far eastward and westward as *An. minimus* (see above), and only *An. minimus* occurs in Taiwan (Chen *et al*., 2002; Somboon *et al*., 2005b) and Assam State of India (Prakash *et al*., 2006; Singh *et al*., 2006). Unfortunately, the synonymy of *An. vincenti* Laveran with *An. minimus* is not so certain. The syntype specimens (adults) of *An. vincenti* were collected at Van Linh in the former French protectorate of Tonkin (Laveran, 1901), which in 1946 formed the northern part of Vietnam bordering on China and is now a Commune in Chi Lang District, Lang Son Province of Vietnam. Electrophoretic studies of the octanol dehydrogenase (*Odh*) enzyme locus and multiplex PCR of ITS2 rDNA indicate that *An. minimus* and species C both occur at this locality (Nguyen Duc Manh, unpublished).

The type series of *An. vincenti* consists of five females mounted in balsam on a single microscope slide (fig. 1). We examined the syntypes of *An. vincenti* and agree with Reid (1947) and Harrison (1980) that two of the five females are specimens of *An. jeyporiensis* James. The other three specimens may be either *An. minimus* or species C. With one possible exception, HP and PSP spots are absent from the wings of these specimens. The possible exception is the questionable presence of faint or partial HP and PSP spots on the left wing of one female. Based on the observations of Van Bortel *et al*. (1999) (see above), there is a slightly greater

likelihood (7.2%) that the three females are specimens of *An. minimus*, but whether one or two or all three specimens are conspecific with *An. minimus* or species C is unanswerable. As indicated above, adults of these species are virtually indistinguishable in northern Vietnam, and molecular methods are unlikely to be useful in resolving their identity because they are mounted in balsam. To resolve this dilemma, the specimen of *An. jeyporiensis* located to the lower right of the other specimens on the microscope slide (fig. 1) is hereby designated the lectotype of *An. vincenti* to unambiguously fix the identity of this nominal species (see figure legend for specific details). As a consequence of this action, *An. vincenti* Laveran, 1901 has priority over *An. jeyporiensis* James, 1902 as the name of the species. Because use of the older synonym will cause taxonomic instability and confusion, we will present a case to the International Commission on Zoological Nomenclature for maintaining the established usage of *An. jeyporiensis*. Until the case has been considered, the junior name of *An. jeyporiensis* should continue to be used as the valid name of the taxon (Article 23.9.3, ICZN, 1999).

The purpose of this paper is to formally name species C of the Minimus Complex and provide information for its identification that will foster further study of its biology in relation to malaria transmission. Species E will be formally named at a later date (P. Somboon, personal communication).

FIGURE 1. The microscope slide bearing the syntypes of *Anopheles vincenti* Laveran. The two specimens indicated by arrows are females of *An. jeyporiensis* James; the other three specimens are females of the Minimus Complex that cannot be identified as either *An. minimus* Theobald or species C of the complex, both of which occur at the type locality of *An. vincenti*. The specimen of *An. jeyporiensis* located at lower right is designated the lectotype of *An. vincenti* (type locality: Van Linh Commune, Chi Lang District, Lang Son Province, Vietnam; depository: Institut Pasteur, Paris [PIP]).

Material and methods

This study is based on specimens of species C collected in sympatry with *An. minimus* in the village of Khoi, Hoa Binh Province, northern Vietnam (*Type series*; table 3).

Morphology. The progeny of five wild-caught females were individually reared to provide adults with associated larval and pupal exuviae. Three broods of *An. minimus* and two of species C were identified by electrophoresis of the octanol dehydrogenase (*Odh*) enzyme locus (Green *et al*., 1990; Van Bortel *et al*., 1999) and PCR-RFLP of ITS2 (Van Bortel *et al*., 2000). Observations of adults were made under simulated natural light. Larval and pupal chaetotaxy were studied using differential interference contrast microscopy. The morphological terminology follows Harbach & Knight (1980, 1982). The specimens are deposited in The Natural History Museum (BMNH), London.

DNA sequences. Sequences for the ITS2, D3, COI and COII of *An. minimus* C collected in the village of Khoi (not included in *Type series*) were published by Garros *et al*. (2005a,b). Sequences for the cytochrome b (Cyt-b) locus of mtDNA are included here to complete the dataset. DNA was extracted from individual specimens stored at –80°C or dried over silica gel following the protocol of Linton *et al*. (2001). The PCR and cycling conditions of Dusfour *et al.* (2004) were used for the amplification of Cyt-b. The six sequences (three per species) for the Cyt-b locus generated in this study are available in GenBank under accession numbers EU071692–EU071694 (*An. minimus*) and EU071695–EU071697 (*An. minimus* C).

Taxonomy

Anopheles **(*Cellia*)** ***harrisoni*** **Harbach & Manguin, sp. n.**

Anopheles minimus species C of Green *et al*., 1990 (enzyme electrophoresis, morphology); Baimai *et al*., 1996 (mitotic karyotype); Sucharit & Komalamisra, 1997 (RAPD-PCR identification); Sharpe *et al*., 1999 (D3 rDNA, ASA and SSCP identification); Sharpe *et al*., 2000 (COII mtDNA, ITS2 rDNA, D3 rDNA, phylogenetic relationships); Van Bortel *et al*., 2000 (ITS2 rDNA, RFLP-PCR assay); Kengne *et al*., 2001 (RAPD-PCR, SCAR-PCR multiplex assay); Somboon *et al*., 2001 (D3 rDNA); Chen *et al*., 2002 (D3 rDNA, morphology, distribution); Choochote *et al*., 2002 (crossmating with *An. minimus*); Rwegoshora *et al*., 2002 (adult bionomics); Zhou *et al*., 2002a (ITS2 rDNA, phylogenetic relationships); Zhou *et al*., 2002b (COII mtDNA, phylogenetic relationships); Chen *et al*., 2003 (COII mtDNA, D3 rDNA, phylogenetic relationships); Phuc *et al*., 2003 (ITS2 rDNA, multiplex assay); Van Bortel *et al*., 2003 (*Odh* locus, population genetics); Garros *et al*., 2004a (D3 rDNA, ITS2 rDNA, RFLP-PCR assay); Garros *et al*., 2004b (ITS2 rDNA, allele specific multiplex assay); Garros *et al*., 2005a (COII mtDNA, D3 rDNA, morphology, phylogenetic relationships); Garros *et al*., 2005b (COI mtDNA, ITS2 rDNA, D3 rDNA, phylogenetic relationships); Trung *et al*., 2004 (trophic behaviour); Van Bortel *et al*., 2004 (trophic behaviour); Kengluecha *et al*., 2005 (larval bionomics); Somboon *et al*., 2005a (crossmating with *An. minimus* E); Trung *et al*., 2005 (trophic behaviour); Harbach *et al*., 2006 (taxonomy); Garros *et al*., 2006 (general review); Potikasikorn *et al*., 2006 (insecticide resistance); Singh *et al*., 2006 (ITS2 rDNA, D2-D3 rDNA); Sungvornyothin *et al*., 2006a (morphology); Sungvornyothin *et al*., 2006b (bionomics) .

Anopheles minimus form II of Van Bortel *et al*., 1999 (*Odh* locus).

Diagnosis. Sequences for the ITS2 and the D3 domain of the 28S rDNA and Cyt-b, COI and COII of mtDNA for *An. harrisoni* and other members of the Minimus Complex exhibit little intraspecific variation and sufficient interspecific variation to be diagnostic of the species (figs 2–6).

Comparative studies of the adult, larval and pupal stages of *An. minimus* and *An. harrisoni* (as *An. minimus* species A and C, respectively) from the type locality of the latter species (see *Type series*) were conducted using specimens from progeny broods identified by enzyme electrophoresis and PCR-RFLP (see **Materials and methods**). Attempts were made to find morphological characters in the various life stages that might differentiate the two species. Study of adult females included the use of scanning electron microscopy to examine the cibarial armature. Study of the larval and pupal stages involved systematic observations of all elements of chaetotaxy. The results of these studies indicated that *An. minimus* and *An. harrisoni* are essentially isomorphic in all life stages (also see Garros *et al*., 2005b). Since no morphological characters were found that would consistently and reliably distinguish these species, studies of their ecology and behaviour must relay on genetic and molecular methods of identification.

Molecular characterization. Three PCR-based assays have been developed that distinguish *An. harrisoni* from *An. minimus* and three related species (*An. aconitus* Dönitz, *An. pampanai* Büttiker, *An. varuna* Iyengar): SSCP-PCR of D3 for identifying four of the five species (Sharpe *et al*., 1999); RFLP-PCR of ITS2 using the restriction endonuclease BsIZI (Van Bortel *et al*., 2000); allele-specific PCR based on SCAR markers (Kengne *et al*., 2001) and ITS2 nucleotide variations (Phuc *et al*., 2003; Garros *et al*., 2004a).

Mean sequence divergence between *An. minimus* and *An. harrisoni* ranged from 2.3 to 3.3% in the Cyt-b region. Levels of variability of the D3 and COII sequences for these two species were reported previously (Garros *et al*., 2005a,b).

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                                10        20        30        40        50        60        70        80        90        10
                          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AY259158 An. minimus      ACGGACCAAGAAGTCTATCTTGCGCGCAAGCCAATGGGTAAATGGTGCGGTACGCTGCCCATGATTGGAAACCCACAGGCGAAGACAAATCGAGTGTTGC
AY259159 An. harrisoni    .......................................................C........C...............AC..................
AJ512721 An. minimus E    .......................................................C........C........T......A...................

                               110       120       130       140       150       160       170       180       190        20
                          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AY259158 An. minimus      GGGATTACGGGTACGGCCGATGGCGCAAGCCTTCGTCGGATCCCTCCATCCCAGGGTGTCCCGTCCGGGTGCTTGCACCCTGTGGACATCCCCGGAGTGC
AY259159 An. harrisoni    ........................................C.......................T...............A.C.................
AJ512721 An. minimus E    ........................................C.......................................A.C.................

                               210       220       230       240       250       260       270       280       290        30
                          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AY259158 An. minimus      GTAGGATGTGACCCGAAAGATGGTGAACTATGCCTGATCAGGTTGAAGTCAGGGGAAACCCTGATGGAGGACCGAAGCAATTCTGACGTGCAAATCGATT
AY259159 An. harrisoni    ....................................................................................................
AJ512721 An. minimus E    ....................................................................................................

                               310       320       330       340
                          ....|....|....|....|....|....|....|....|.
AY259158 An. minimus      GTCAGAGTTGGGCATAGGGGCGAAAGACCAATCGAACCATC
AY259159 An. harrisoni    .........................................
AJ512721 An. minimus E    .........................................
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FIGURE 2. Alignment of the 28S sequences (341 bp) of *Anopheles minimus*, *An. harrisoni* and species E of the Minimus Complex.

Holotype female. ADULT: Exactly as the neotype series of *An. minimus* described by Harbach *et al*. (2006), except as follows. *Head*: Proboscis length about 1.6 mm. Maxillary palpus 1.5 mm long, apical pale band slightly longer than preapical dark and pale bands of equal length. *Thorax*: Pleura with 2 prespiracular, 2 prealar, 2 upper mesokatepisternal and 5 upper mesepimeral setae. *Wing*: Length 2.9 mm; costa with humeral pale spot in addition to presector pale, sector pale, subcostal and preapical pale spots, sector and accessory sector pale spots of R fused; distal 0.25 of 1A pale-scaled; pale fringe spots fused at apices of R_3 and R_{4+5} (total of 6 pale fringe spots). PUPAL EXUVIAE: Habitus and chaetotaxy as described and illustrated for *An. minimus* by Harbach *et al*. (2006), except as follows; number of branches of setae in table 1. *Cephalothorax*: Seta 4-CT with 7/5 branches (1–3 in *An. minimus*) [Harrison (1980) observed 7-10 branches in specimens from Thailand that may have included *An. harrisoni*][1]; 8-CT forked on one side (single in *An. minimus*). *Trumpet*: Length 0.35 mm, meatus 0.10 mm, pinna 0.35 mm. *Abdomen*: Length 2.33 mm. Seta 5-I with 1/2 branches (2–4 in *An. minimus*); 7-I with 6/7 branches (4–6 in *An. minimus*) [3–7 branches in Thai specimens]; 9-I with 6/8 branches (2–5 in *An. minimus*); 0-IV with 5/4 branches (2–4 in *An. minimus*) [1–6 branches in Thai specimens]; 4-IV with 3/5 branches (2,3 in *An. minimus*) [1–6 branches in Thai specimens]; 8,10,11-II absent. *Genital lobe*: Longer, length 0.20 mm. *Paddle*: Length 0.67 mm, width 0.43 mm, index 1.56; marginal serrations begin 0.15 from base and end 0.47 from base; refractile index 0.31. LARVAL EXUVIAE (fourth-instar): Habitus and chaetotaxy as described and illustrated for *An. minimus* by Harbach *et al*. (2006), except as follows; number of branches of setae in table 2. *Head*: Width 0.55 mm, length 0.59 mm. Seta 7-C with 19/

1. From this point onward, information in square brackets refers to data of Harrison (1980).

15 branches (14–19 in *An. minimus*) [15–20 in Thai specimens]; 8-C with 8/9 branches (4–7 in *An. minimus*) [5–10 in Thai specimens]; 8-P with 35/? branches (31–34 in *An. minimus*) [27–38 in Thai specimens]. *Antenna*: Length 0.23 mm. *Thorax*: Mesothorax without pair of submedian notal plates. Seta 8-P with 35/? branches (31–34 in *An. minimus*) [27–38 in Thai specimens]; *Abdomen*: Segments I–VII with distinct submedian accessory tergal plates. Seta 0-III with 3/2 branches (1,2 in *An. minimus*) [1–3 in Thai specimens]; 1-III,V with 23/22 and 22/20 branches, respectively (16–22 and 16–21 in *An. minimus*) [17–25 and 17–22 in Thai specimens]; 4-I,II with 9/9 and 7/9 branches, respectively (both 4–7 in *An. minimus*) [4–8 and 5–9 in Thai specimens]; 5-VI,VIII with 8/9 and 7/7 branches, respectively (9–13 and 4–6 in *An. minimus*) [9–12 and 5–7 in Thai specimens]; 6,7-I both with ?/34 branches (23–31 and 22–33 in *An. minimus*) [26–34 and 26–33 in Thai specimens]; 7-II with 36/39 branches (22–33 in *An. minimus*) [24–36 in Thai specimens]; 7-IV with 10/7 branches (5–7 in *An. minimus*) [4–8 in Thai specimens]. Pecten plate with 13/12 spines. Saddle length 0.23 mm. Seta 4-X with 4–14 branches (4–13 in *An. minimus*).

TABLE 1. Number of branches for pupal setae (left/right sides) of the holotype female of *Anopheles harrisoni*. Question marks indicate missing setae.

Setae	Cephalothorax	Abdominal segments									Paddle
no.	CT	I	II	III	IV	V	VI	VII	VIII	IX	P
0	–	–	1/1	3/?	5/4	3/2	1/2	1/1	1/1	–	–
1	4/3	nc	24/30	18/21	13/12	2/1	1/1	1/1	–	3/4	1/1
2	2/3	5/6	5/6	9/7	9/9	5/7	6/6	5/4	–	–	5/?
3	5/6	2/2	7/7	6/6	7/7	1/2	1/1	3/1	–	–	–
4	7/5	8/7	6/5	5/3	3/5	4/4	1/1	1/1	3/2	–	–
5	8/6	1/2	6/6	10/9	9/9	6/5	6/6	4/5	–	–	–
6	4/6	2/2	1/1	?/6	4/5	3/3	2/1	1/2	–	–	–
7	2/2	6/7	5/4	3/4	4/3	5/5	1/1	1/1	–	–	–
8	1/2	–	–	4/4	3/?	2/1	2/2	3/3	–	–	–
9	3/4	6/8	1/1	1/1	1/1	1/1	1/1	1/1	8/9	–	–
10	2/2	–	–	3/3	2/1	1/1	2/1	3/2	–	–	–
11	5/4	–	–	1/1	1/1	1/1	1/1	2/1	–	–	–
12	5/3	–	–	–	–	–	–	–	–	–	–
14	–	–	–	1/1	1/1	1/1	1/1	1/1	1	–	–

nc = not counted.

Systematics. *Anopheles harrisoni*, like *An. minimus*, is very similar to three other species of the Myzomyia Series that occur within its range of distribution in the Oriental Region, i.e. *An. aconitus*, *An. fluviatilis* James and *An. varuna* (see e.g. Van Bortel *et al*., 2001). As pointed out by Harrison (1980), no morphological characters are completely reliable for distinguishing the adults of these species. Furthermore, the adults of *An. pampanai* are also often misidentified as *An. minimus*, and hence *An. harrisoni*, because the distinguishing features of the wings are not easily discerned. Consequently, adults of *An. harrisoni* (as well as those of other members of the Minimus Complex), cannot be distinguished from the adults of these species with certainty without associated larval and pupal exuviae. The morphological characters in the identification keys of Harrison (1980) and Rattanarithikul *et al*. (2006) that distinguish *An. minimus* from the closely related species also distinguish *An. harrisoni* from those species. However, because of the uncertainties associated with morphological differentiation, the various types of molecular assays developed by Sharpe *et al*. (1999), Van Bortel *et al*. (2000), Kengne *et al*. (2001), Phuc *et al*. (2003) and Garros *et al*. (2004a,b) should be used for the unequivocal identification of *An. harrisoni* (= their *An. minimus* species C).

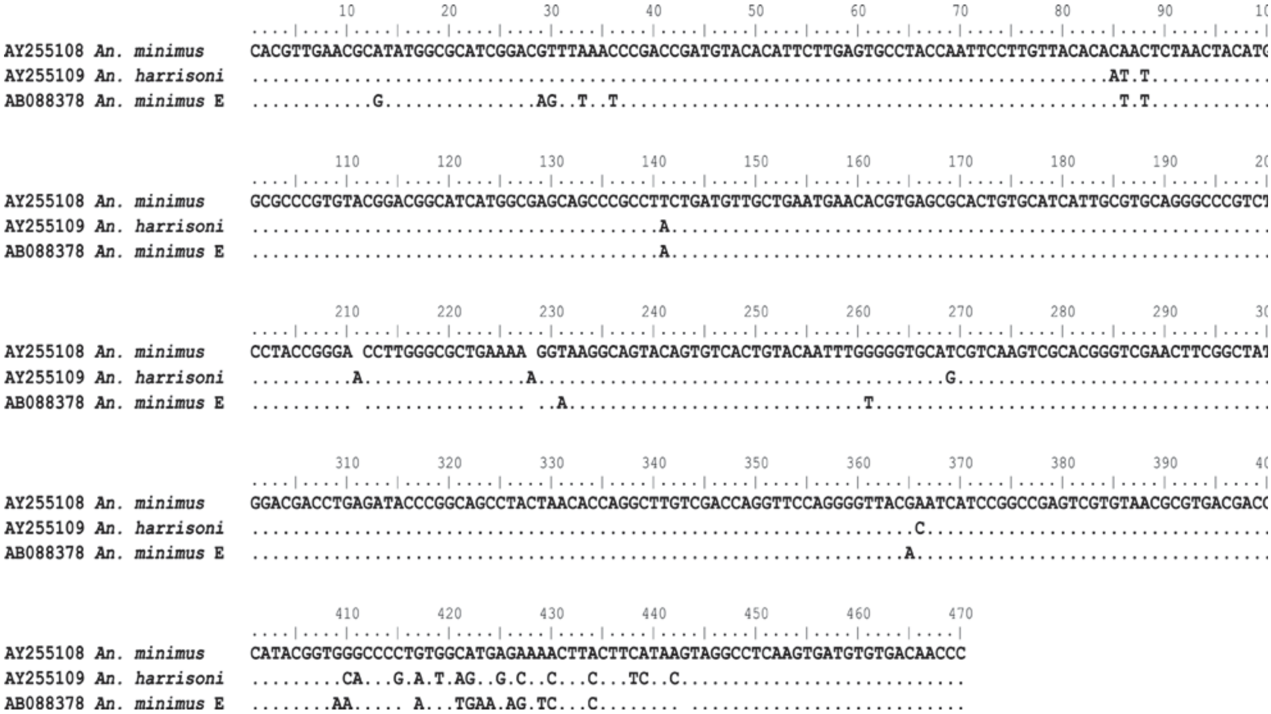

FIGURE 3. Alignment of the ITS2 sequences (470 bp) of *Anopheles minimus*, *An. harrisoni* and species E of the Minimus Complex.

TABLE 2. Number of branches for larval setae (left/right sides) of the holotype female of *Anopheles harrisoni*. Question marks indicate missing setae or setae with damaged or obscured branches.

Setae	Head	Thorax			Abdominal segments								
no.	C	P	M	T	I	II	III	IV	V	VI	VII	VIII	X
0	1/1	1/1	–	–	–	1/1	3/2	3/3	3/2	2/2	2/2	2/2	–
1	1/1	22/22	31/33	3/2	16/14	18/20	23/22	21/22	22/20	20/19	16/17	2/2	1/1
2	1/1	14/16	2/1	1/1	5/7	5/5	3/3	1/1	1/1	1/1	3/3	10/11	20/20
3	1/1	1/1	1/1	15/14	1/1	1/1	1/1	3/3	1/1	1/1	3/3	?/10	11/8
4	1/1	12/13	5/?	4/4	9/9	7/9	5/5	4/5	2/2	1/1	?/1	4/4	4–14*
5	15/14	30/29	1/1	?/33	5/5	6/4	6/5	6/4	8/?	8/9	?/10	7/7	–
6	15/15	1/1	3/3	5/4	?/34	26/28	16/18	3/3	3/3	3/3	4/3	–	–
7	19/15	26/25	3/4	27/34	?/34	36/39	7/8	10/7	7/7	5/5	?/5	1-S,	9/9
8	8/9	35/?	19/26	32/?	–	3/3	2/3	3/3	2/3	3/3	6/8	2-S,	8/?
9	6/7	12/?	1/1	6/5	6/6	6/9	6/7	7/7	7/8	9/6	9/9	6-S,	3/3
10	3/3	1/1	1/1	1/1	5/5	3/3	3/2	3/3	3/3	3/3	6/4	7-S,	1/2
11	44/?	3/?	1/1	1/1	4/5	2/4	4/3	4/3	4/4	4/4	3/3	8-S,	7/?
12	4/4	1/1	2/2	3/?	4/5	5/4	4/3	2/4	2/4	4/2	?/2	9-S,	5/5
13	6/?	6/7	6/8	4/3	7/9	12/10	5/5	5/5	5/5	11/9	4/4	–	–
14	5/?	5/5	8/9	–	–	–	1/2	1/1	2/2	2/2	3/2	2	–
15	?/?	–	–	–	–	–	–	–	–	–	–	–	–

*Range of branches for individual setae (9 pairs).

Bionomics. Little specific bionomical information is available for *An. harrisoni* because this species was not distinguished from *An. minimus* during the course of ecological and epidemiological studies conducted before, and even after, the advent of molecular methods of identification. The trophic behaviour and seasonality of *An. harrisoni* (as *An. minimus* C) has been examined in northern Vietnam and western Thailand (Rwegoshora *et al*., 2002; Van Bortel *et al*., 2004; Trung *et al*., 2005, Sungvornyothin et al., 2006b) where this species occurs in sympatry with *An. minimus*, but its larval ecology and biology are still unknown. Both species may inhabit the same larval habitats (Garros *et al*., 2006). In northern Vietnam (Hoa Binh Province), adults of *An. harrisoni* were particularly abundant in October during the dry season (Van Bortel, 2002; Garros *et al*., 2006). In western Thailand, a two-year survey showed that populations of the species peaked in April–June and November–December, which corresponds with the beginning and the end of the rainy season (Sungvornyothin *et al*., 2006b). In contrast, an earlier one-year study showed that populations in the same area of Thailand exhibited peak biting density at the end of the rainy season in October–November and a second, smaller peak during the latter part of the dry season in January–March (Rwegoshora *et al*., 2002). The density of *An. harrisoni* was exceptionally high at sites in western Thailand (Ban Phu Toei, Sai Yok District, Kanchanaburi Province) and central Vietnam (Lang Nhot Village, Khanh Phu Commune, Khanh Vinh District, Khanh Hoa Province) (Rwegoshora *et al*., 2002; Garros *et al*., 2005c; Kengluecha *et al*., 2005; Sungvornyothin *et al*., 2006b). Two biting peaks were observed indoors in Thailand, one around 1900 h and another after midnight (around 0100 h) (Sungvornyothin *et al*., 2006b). Only the later peak was recorded outdoors. The relative risk of being bitten before 2200 h was higher for *An. harrisoni* than for *An. minimus*, which exhibited peak feeding activity after 2200 h in Vietnam (Trung *et al*., 2005).

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                                10        20        30        40        50        60        70        80        90       100
                        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AY423058 An. minimus    GGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCCAGATATAGCATTCCCTCGAATAAATAATATAAGATTTTGAATACTTCCTCCTTCTT
AY423057 An. harrisoni  ............................................................................................A.......

                               110       120       130       140       150       160       170       180       190       200
                        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AY423058 An. minimus    TAACTCTTCTTATTTCTAGAAGTATAGTAGAAAATGGAGCAGGAACTGGTTGAACTGTTTATCCTCCTCTATCTTCTGGGATTGCTCATGCTGGAGCTTC
AY423057 An. harrisoni  ...................................................................C.....A.....A..............G.....

                               210       220       230       240       250       260       270       280       290       300
                        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AY423058 An. minimus    AGTAGATTTAGCTATTTTTTCACTACATTTAGCTGGGATTTCTTCTATTTTAGGGGCAGTAAATTTTATTACTACTGTTATTAATATACGATCACCAGGA
AY423057 An. harrisoni  ......................................................A......................................G......

                               310       320       330       340       350       360       370       380       390       400
                        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AY423058 An. minimus    ATTACATTAGATCGAATACCTCTTTTTGTATGATCTGTAGTAATTACTGCTATTTTATTATTATTATCTTTACCTGTTTTAGCTGGGGCTATTACAATAT
AY423057 An. harrisoni  ...................................A..............C................................G..A.............

                               410       420       430       440       450       460       470       480       490       500
                        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AY423058 An. minimus    TACTAACTGATCGAAATCTTAATACTTCATTCTTTGACCCAGCAGGTGGAGGAGATCCTATTTTATATCAACATTTATTTTGATTTTTTGGACACCCAGA
AY423057 An. harrisoni  ..T.................................................................................................

                               510       520
                        ....|....|....|....|....
AY423058 An. minimus    AGTTTATATTTTAATTTTACCGGG
AY423057 An. harrisoni  ........................
```

FIGURE 4. Alignment of the COI sequences (524 bp) of *Anopheles minimus* and *An. harrisoni*.

Studies of trophic behaviour have shown that *Anopheles harrisoni* is more zoophilic than anthropophilic, and is exophagic and exophilic in both northern Vietnam and western Thailand (Rwegoshora *et al*., 2002; Van Bortel *et al*., 2004; Trung *et al*., 2005; Sungvornyothin *et al*., 2006b). It will not be surprising if future studies find that the feeding behaviour of *An. harrisoni* is as highly variable as that of *An. minimus*, which is known to be an opportunist feeder (Van Bortel *et al*., 2004; Trung *et al*., 2005). The vectorial status of *An. harrisoni* has not been determined, but evidence suggests that it is a major vector of malaria in southern China (Chen *et al*., 2002).

TABLE 3. Comparison of members of the Minimus Complex: species, source localities (Pr.: Province, Arch.: Archipelago), GenBank accession numbers and DNA fragments (alignment lengths in parentheses). Sequence data for *An. harrisoni* are based on specimens collected at the same locality as the type specimens. Sequences for the 28S/D3, ITS2, COI and COII loci were published previously by Garros *et al*. (2005a,b). Cyt-b sequences were obtained in the present study. The sequences marked with an asterisk (*) were obtained from GenBank. na = not available.

Species	Localities	Latitude/longitude	Accession no.				
			28S/D3 (341 bp)	ITS2 (470 bp)	COI (524 bp)	COII (631 bp)	Cyt-b (711 bp)
An. harrisoni	Vietnam, Hoa Binh Pr., Khoi Village	20°38’12” N/ 105°10’2”E	AY259159	AY255109	AY423057	AY486111	EU071695–7
An. minimus	(See above)	(See above)	AY259158	AY255108	AY423058	AY486110	EU071692–4
An. minimus E	Japan, Ryukyu Arch., Ishigaki Island	24°26’ N/ 124°11’ E	AJ512721*	AB088378*	na	AJ512739*	na

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                                10        20        30        40        50        60        70        80        90       100
                        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AY486110 An. minimus    ATGGCAACATGAGCAAATTTAGGATTGCAAGATAGATCATCCCCTTTAATAGAACAATTAAATTTTTTCCACGATCATACATTATTAATTTTAACAATAA
AY486111 An. harrisoni  .........................................A..........................T...............................
AJ512739 An. minimus E  .........................................T..........................T.................G.............

                               110       120       130       140       150       160       170       180       190       200
                        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AY486110 An. minimus    TTACAATTTTAGTTGGATATATTATAGGAATATTATTATTTAATAAATTTACTAATCGATATTTACTTCACGGACAAACTATTGAAATTATTTGAACTGT
AY486111 An. harrisoni  ....................................................................................................
AJ512739 An. minimus E  .......................................................C............................................

                               210       220       230       240       250       260       270       280       290       300
                        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AY486110 An. minimus    ATTACCAGCAATTATTTTAATATTTATTGCTTTTCCTTCTTTACGATTATTATATTTAATAGACGAAATTAATACACCTTCTATTACTTTAAAATCAATT
AY486111 An. harrisoni  .................................C....................C.............................................
AJ512739 An. minimus E  ..............................C.....................................................................

                               310       320       330       340       350       360       370       380       390       400
                        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AY486110 An. minimus    GGACATCAATGATATTGAAGTTACGAATATTCAGATTTTTAAATTTAGAATTTGACTCATATATAATCCCAACAAACGAACTTGAAACAAATGGATTCC
AY486111 An. harrisoni  ....................C........C......................................................................
AJ512739 An. minimus E  ....................................................................T........T....................T.

                               410       420       430       440       450       460       470       480       490       500
                        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AY486110 An. minimus    GATTATTAGATGTTGATAATCGTATTGTTTTACCTATAAATAATCAAATTCGAATTTTAGTTACAGCAACAGATGTATTACATTCTTGAACAGTTCCTTC
AY486111 An. harrisoni  ......................A...............................................T......C......................
AJ512739 An. minimus E  ......................A...............................................C.............................

                               510       520       530       540       550       560       570       580       590       600
                        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AY486110 An. minimus    TTTAGGAGTAAAGGTTGATGCAACACCGGGACGATTAAATCAAATTAACTTTTAATTAATCGACCAGGATTATTTTTGGGCAATGTTCAGAAATTTGT
AY486111 An. harrisoni  ......G....................A......C.............T........C.......................A..................
AJ512739 An. minimus E  ...........................T....................T................................A.....C............

                               610       620       630
                        ....|....|....|....|....|....|.
AY486110 An. minimus    GGAGCAAATCATAGATTTATACCAATTGTAA
AY486111 An. harrisoni  ...............................
AJ512739 An. minimus E  ...............................
```

FIGURE 5. Alignment of the COII sequences (631 bp) of *Anopheles minimus*, *An. harrisoni* and species E of the Minimus Complex.

Distribution. Current data show that *An. minimus* is the predominant species of the Minimus Complex in the Oriental Region. It is recorded from northeastern India to eastern China (Taiwan) and southward from Sichuan Province of China through Laos, Thailand, Vietnam and Cambodia (Subbarao, 1998; Van Bortel *et al*., 1999, 2000; Kengne *et al*., 2001; Chen *et al*., 2002; Somboon *et al*., 2005b). In comparison, available records indicate that *An. harrisoni* has a smaller, patchy distribution in Southeast Asia. Populations have been

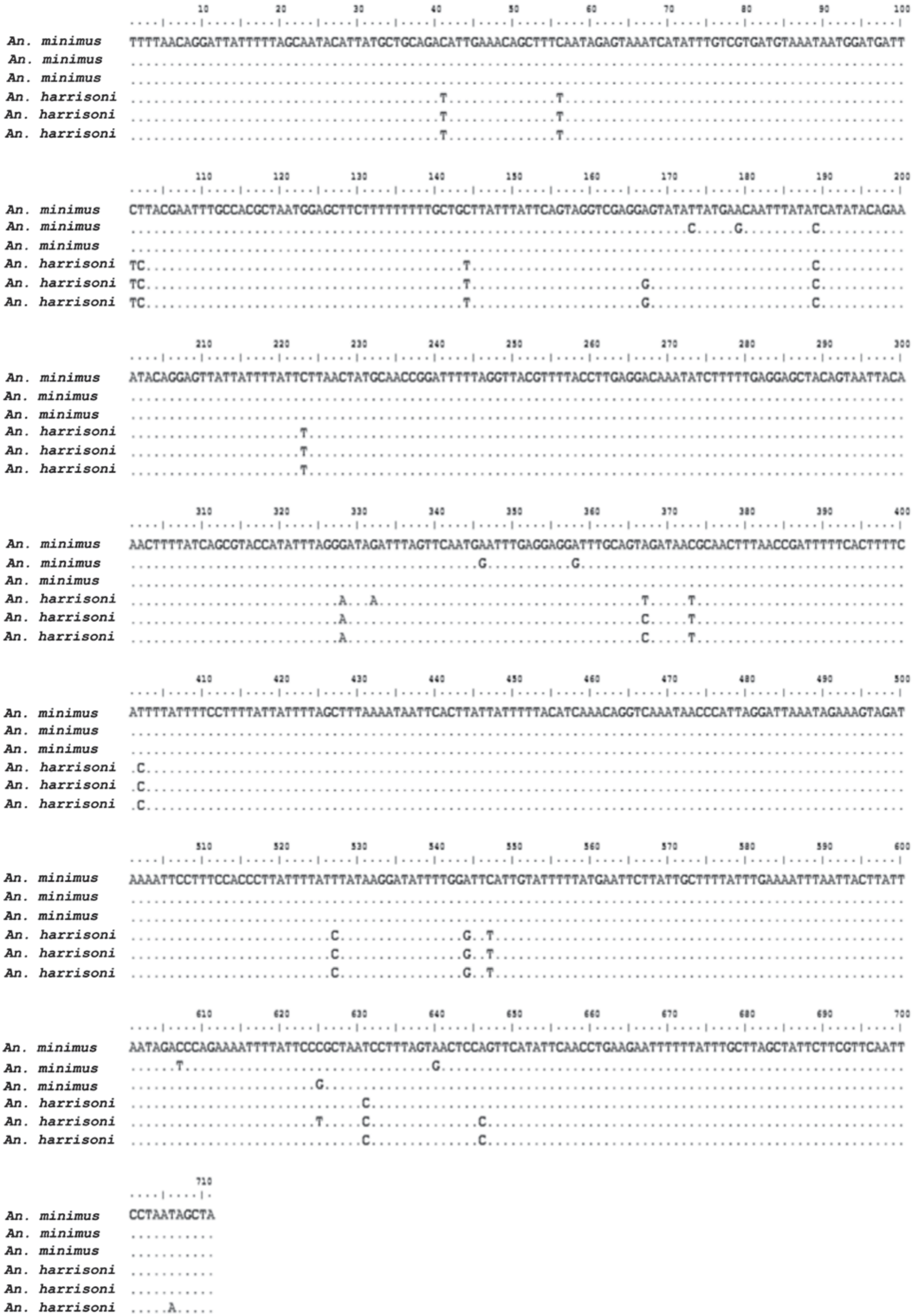

FIGURE 6. Alignment of the Cyt-b sequences (711 bp) of *Anopheles minimus* and *An. harrisoni*.

documented in south-central China (Chen *et al*., 2002), central Myanmar (Singh *et al*., 2006), northern and central Vietnam (Van Bortel *et al*., 1999, 2000; Kengne *et al*., 2001; Garros *et al*., 2005c) and northwestern Thailand along the Thai-Myanmar border (Green *et al*., 1990; Sharpe *et al*., 1999; Rattanarithikul *et al*., 2006; Singh *et al*., 2006; Sungvornyothin *et al*., 2006a,b). Whether *An*. *harrisoni* occurs in areas between these disjunctive localities, i.e. Laos, central and eastern Thailand, and Cambodia, is unknown.

Etymology. This species is named in honor of Dr. Bruce A. Harrison (Public Health Pest Management, North Carolina Department of Environment and Natural Resources, Winston-Salem, North Carolina) for his many important contributions to our knowledge of *Anopheles* mosquitoes in the Oriental Region, especially his taxonomic investigations of the Myzomyia Series (Harrison, 1980) that provided the foundation for integrated morphological and molecular studies of this medically important group of insects.

Type series. One hundred and sixty-five specimens from 2 progeny broods (18 females [♀], 17 males [♂], 65 larval exuviae [Le], 65 pupal exuviae [Pe]). *Holotype*, ♀ (HB4-14), with LePe on microscope slide, VIETNAM: Hoa Binh Province, Tan Lac District, Phu Cuong Commune, village of Khoi, 10.ix.1999 (*NIMPE staff*) (BMNH). *Paratypes*, 17♂LePe (HB4-11, -13, -15, -17 through -20; HB5-5, -8, -9, -11 through -14, -17 through -19), 17♀LePe (HB4-2, -4 [head and cibarium on SEM stub], -5, -7, -9, -10, -12 [head and cibarium on SEM stub]; HB5-1 through -4, -6, -7, -10, -15 [head and cibarium on SEM stub], -16, -20 [head and cibarium on SEM stub]), 65LePe (HB4-3, -21 through -41; HB5-21, -24 through -30), same data as holotype (BMNH).

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